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The Action of Digitoxin on Some of the Chemical Constituents of the Dog Myocardium

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THE ACTION OF DIGITOXIN ON SOME OF THE CHEMICAL CONSTITUENTS OF THE DOG MYOCARDIUM

by

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LIFE

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CHAPTER I

INTRODUCTION AND THE STATEMENT OF THE PROBLEM

The mechanism of cardiac failure in man is as yet obscure. Perhaps the ideal approach to the solution of this problem is to produce heart failure in experimental animals and to investigate various aspects of the altered physiology. Up to the present time, the production of chronic failure in animals, which would resemble failure in man, has been difficult to achieve.

A second approach to the problem does not a priori require experimental animals in cardiac failure. This procedure takes cognizance of the fact that the administration of a cardiac glycoside improves the failing heart in man. By introducing this drug into normal animals, any change in physiology may suggest the mechanism of action of the drug and, simultaneously, the mechanism of cardiac failure. This will be the procedure which will be followed in this investigation.

One attractive hypothesis with regard to the mechanism of failure is that there is an alteration in energy metabolism. For example, certain investigators have felt that there

is a decrease in the rate of formation of energy-yielding compounds of the heart. Others feel that the utilization of available energy is impaired in the failing heart. An examination of the concentration of certain important chemical constituents may give us a clue with regard to the change in altered metabolism. It will be the purpose of this study to execute such analytical procedures in digitoxin treated animals.

It is important to note that the administration of therapeutic amounts of the drug may not result in an immediate physiological change. This may, in fact, account for negative results which have been reported in the study of the effect of therapeutic amounts of cardiac glycosides on phosphate compounds of the heart (Wollenberger, 1951). In order to avoid overlooking this possibility, it may be necessary to study animals which have been treated with a cardiac drug over several days. In the present investigation animals given daily doses will be studied along with animals given just single doses of the drug.

Finally, it is important to choose for study the chemical constituents which will yield the most meaningful results. The most important compounds to study in this regard may be the so called energy-rich phosphate compounds. Adenosine triphosphate (ATP) and phosphocreatine (PC) have been shown to play important roles in the contraction process not only in skeletal muscle (Sandow et. al., 1947; Szent-Györgyi, 1947) but in cardiac muscle

as well (Cruickshank, 1936; Olson and Schwartz, 1951). ATP is considered to be the immediate source of chemical energy for muscular contraction and is acted upon by an enzyme which releases energy by the hydrolysis of the high energy phosphates. Resynthesis of ATP and of PC, which functions as a reservoir of energy-rich phosphates, takes place during recovery.

A second chemical constituent which is important in enzymatic processes within the cell is potassium. It has been shown that potassium specifically increases the rate of creatine phosphorylation (Boyer et. al., 1943). The presence of potassium is necessary in many enzyme systems which form ATP.

Muscle glycogen is an important emergency source of chemical energy for the production of energy-rich phosphates, perhaps more so in cardiac muscle than in skeletal muscle (Soskin and Levine, 1952). It was felt that lactic acid should also be included among the chemical constituents to be analyzed because it is known that breakdown of glycogen results in accumulation of lactic acid in cardiac failure.

Statement of the Problem.

Since exposure to cardiac glycosides for short periods of time may not measurably alter the metabolism of the heart, it was felt that an investigation using repeated doses over a long period of time as well as single doses would be valuable in the study of the mechanism of action of the drug. Since

previous workers have investigated only one chemical constituent at a time, it appeared that a comprehensive study of inorganic phosphate, energy-rich phosphates, carbohydrates, and electrolytes on the same normal dog hearts would aid in a better understanding of the action of cardiac glycosides on the mammalian heart.

CHAPTER II

REVIEW OF LITERATURE

A. Preliminary Remarks.

Controversal statements may be found in the literature pertaining to the action of cardiac glycosides on some of the chemical constituents, namely inorganic phosphate (IP), energy-rich phosphates, glycogen, lactic acid, and electrolytes (Weicker, 1935; Wollenberger, 1949; Cherkes, 1940; Liebig, 1940; Kimura, 1948; Hagen, 1939; Boyer and Poindexter, 1940; Calhoun and Harrison, 1931; Wedd, 1939). Although there may not be actual agreement between the results of various investigators, it must be realized that the experimental conditions, methods of analysis, and the amount of the glycoside given vary with the investigator.

B. Determination of Dosage.

Dearing et. al. (1943) have performed extensive studies with cardiac glycosides in dogs and cats. The method of Hatcher and Brody (1910) was used to evaluate the minimal lethal dose (MLD) which was found to be 0.42 mg./kg. in the cat and 0.575 mg./kg. in the dog. It was found that after administration

of a single or divided doses of 30% MLD, given over a period of 48 hours, no myocardial lesions appeared. Histological studies were made after a minimum of 6 days and a maximum of 56 days. Sixty per cent MLD when given as a single dose produces definite myocardial lesions and that the frequency of lesions occurring increased as the size of a single dose was increased to 60% MLD. These lesions were not found until 5 or more days after the injection. The authors concluded that a multitude of chemical changes may occur before any demonstrable morphologic changes are observed.

In their experiments on coronary flow in dogs they used 70% of MLD as a calculated toxic dose and 30% of MLD as a calculated therapeutic dose. The results of these experiments show that therapeutic doses of digitalis did not produce a significant change in coronary flow whereas toxic doses decreased the coronary flow. Myocardial lesions were observed in animals in which the coronary flow was kept below the normal level for 12 days by repeated injections.

Wood and Moe (1942) in their studies have accepted as a standard for the doses of the cardiac glycosides the definition of Moe and Visscher (1939). This definition states that a toxic dose of digitalis is one which produces cardiac arrhythmias in the heart of the heart-lung preparation, while a therapeutic dose is one which will increase the external me-

chanical efficiency of the heart-lung preparation without the production of cardiac arrhythmias within a period of 150 minutes after the administration of the drug.

Studies have been made on electrocardiographic changes after therapeutic and toxic doses of cardiac glycosides. The main purpose of these studies has been to use electrocardiographic change as a criterion of digitalis intoxication. In 1923, Pardee from his studies on human subjects has concluded that the changes in T waves could be used as a measure of the minimal effective dose. His work was refuted by Brahms (1929), who studied the effects of various preparations of digitalis in therapeutic and toxic doses on dogs and cats. Since constant depression of T wave did not occur and no negative T wave resulted Brahms has opposed Pardee's suggestion to use the T wave as a criterion for the effect of cardiac glycosides. Brahms' conclusion has been supported by further studies (Brahms and Gaberman, 1931; Schwartz and Weiss, 1929). On the other hand, Bromer and Blumgart (1929) have agreed with Pardee. In 1943, Dearing et. al. after thorough investigations have also concluded that T wave change is not a criterion for digitalis intoxication, since the changes of T wave followed the same pattern after administration of therapeutic as well as toxic doses.

A sign of nausea and vomiting has been accepted by some of investigators as a criterion for a toxic dose (Calhoun

and Harrison, 1931).

A study of the influence of digitalis in the contraction of isolated cardiac muscle in mammals has been reported by Cattell and Gold (1938). Isolated papillary muscles of cat have been used in these experiments. After therapeutic doses of cardiac glycosides there appears to be an increase of the force of cardiac muscle contraction. The primary objection to this preparation is that the dose under these conditions may differ from the dose to be given an intact animal.

C. Energy Utilization and Energy Formation.

The most striking effect of cardiac glycosides on the failing heart is the strengthening of its contractile power. In the normal heart contractility appears not to be markedly altered unless toxic doses are given. Starling and co-workers (1914) have laid the basis for the study of the energetics of the failing heart by demonstrating that the mechanical energy freed in the contraction of the heart depends upon its diastolic volume. This is the well known Law of the Heart. At a given diastolic volume the failing heart, having a smaller capacity to perform work has to increase its diastolic volume.

Under constant conditions, the oxygen consumption of the heart, which is a measure of total energy utilization, is determined by its diastolic volume not only in the normal heart, but also in the spontaneous failure of the heart-lung preparation.

As the heart is failing, the oxygen consumption increases in order to maintain the same amount of work (Starling and Visscher, 1927), or the oxygen consumption remains the same, when the diastolic volume is constant and the work decreases. In both cases the mechanical efficiency is decreased. These findings and conclusions have been confirmed by Moe and Visscher (1939). They have, however, been disputed by Katz (1936), who has reported no loss of mechanical efficiency in failure. Katz believes that the impairment of energy formation may be the basis for failure.

D. Action of Cardiac Glycosides on Energy-Rich Phosphates.

The importance of PC and ATP in the contraction of skeletal and cardiac muscle and its association with the transfer of chemical energy into mechanical energy has been accepted for a number of years. To date there appears to be no unanimity of opinion on the effect of cardiac glycosides on these phosphate fractions.

Using non-toxic doses of digitoxin, there were no marked changes in PC and ATP in the intact animal (Kimura, 1948; 12 mg./kg. in the rat; Wollenberger, 1951).

Using therapeutic doses of cardiac glycosides, Wollenberger (1948) found in the relatively non-failing heart-lung preperation no significant change in ATP and PC content. In spontaneous failure occurring in a heart-lung circuit the PC and

ATP fractions also remain essentially normal (Wollenberger, 1947).

In the isolated Langendorff preparation perfused with Ringer's solution there was no marked change in PC and ATP with non-toxic doses (0.03-0.05 mg./kg. strophanthin, Weicker, 1935). Spontaneous failure of the mammalian heart perfused with Locke solution is associated with a decline in oxygen consumption, PC and possibly ATP (Rohde, 1912; Mgge, 1933; Weicker, 1935); administration of a cardiac glycoside (strophanthin) results in a return to normal contractility with a corresponding increase in oxygen consumption (Weicker, 1935). Under such condition PC and ATP is restored to normal.

In spontaneous failure induced by anesthetics in the heart-lung preparation noticeable changes in PC and ATP are not produced by non-toxic doses of cardiac glycosides (Wollenberger, 1949).

With regard to toxic dose of the drug, Chen and co-workers (1947) found that the toxic effect of cardiac glycosides on mammalian hearts, in its more advanced stages, is characterized by a depletion of the energy-rich phosphate store. Giving either digitoxin or ouabain infusions to dogs (0.05 mg./kg., Wollenberger, 1951) a marked depletion of PC occurs with the onset of ventricular fibrillation, while the ATP content is not significantly altered. The same results have been found in the heart-lung preparation of the heart poisoned with ouabain or

digoxin (Wollenberger, 1948). In the isolated cat heart perfused with Ringer's solution, contracture-producing doses of strophanthin cause a 50% loss of PC and even a greater reduction of ATP (0.1-0.15 mg./kg. strophanthin, Weicker, 1935).

To summarize these studies it can be said that a non-toxic dose of cardiac glycosides does not appear to alter the concentration of PC and ATP in intact animal as well as in the non-failing heart-lung or isolated heart preparation. Poisoning the heart in either of these preparations with such drugs causes a marked depletion of the energy-rich phosphates. It appears that in no study has the action of the drug been studied over a time period longer than several hours, with the exception of Kimura's work (1948).

E. Action of Cardiac Glycosides on Glycogen and Lactic Acid.

Following the administration of non-toxic doses of cardiac glycosides, the glycogen content of the heart has been reported to be increased (rabbit, Cherkes, 1940), decreased (rabbit, Liebig, 1940), or not significantly changed (rat, Kimura, 1948). In the rat experiments 12 mg./kg. per day of digitoxin in propylene glycol was given intraperitoneally for three days.

All the previously mentioned authors maintain that the glycogen stores in heart, liver, and skeletal muscle are depleted in animals poisoned with cardiac glycosides. A decrease

in the glycogen content of the hearts of rats and dogs acutely poisoned with digitoxin and strophenthin is associated with a correspondingly sharp rise in lactic acid (Cherkes, 1940).

Glucose and lactic acid utilization by the heart in a freshly prepared as well as in a spontaneously deteriorating heart-lung preparation is markedly increased, at constant work levels, by cardiac glycosides (Grunal, 1940; Mel'nikova, 1942).

An increased uptake of lactic acid but not pyruvic acid in an isolated frog heart in a medium containing digilanid was observed by Mardones (1948). The author concluded since the oxygen uptake remain unchanged that the extra lactate consumed is not oxidized, but is converted to glycogen.

The literature thus appears to indicate that glycogen utilization may be increased, decreased, or unchanged, and lactic acid utilization may be increased by therapeutic doses of cardiac glycosides. An increase in lactic acid production may be expected, particularly as toxic levels of the drug are reached.

F. Action of Cardiac Glycosides on Electrolytes.

The experimental results obtained by administering therapeutic doses of digitalis on the potassium content of the heart are controversial. Also, there is much discrepancy in the literature on the electrolyte content of the heart in patients dying of heart failure.

A slight decrease of questionable significance or no change in the potassium content of the intact dog and intact cat myocardium has been shown after the administration of a therapeutic dose of the cardiac glycoside by Calhoun and Harrison (1930) and by Wedd (1941) respectively. These findings, however, are not supported by Boyer and Poindexter (1940), who have reported that therapeutic dosages of digitalis increase the potassium content of the hearts of intact cats. In their experiments 11 cats were used, 5 of which were digitalized with toxic doses of digifoline on the day of the experiment. The other 6 cats received intraperitoneally daily therapeutic doses of digitalis over a period of 2 to 5 days.

After the administration of therapeutic doses of digitalis the blood findings of Wood and Moe (1942) indicate a loss of potassium from the heart or lung in a heart-lung preparation. The cardiac potassium of Langendorff perfused rabbit heart increases after therapeutic doses of digitoxin ranging from 3.6 to 4.6 mg./gm. of weight of heart (Hagen, 1939), while Wedd (1941) could demonstrate no significant change in the potassium content in isolated strips of turtle heart. These conflicting results are difficult to explain simply unless species difference or the difference in preperation can be involved.

Toxic doses of digitalis cause a decrease in the potassium concentration of the ventricular musculature of the heart-

lung preparation (Wood and Moe, 1938), the Langendorff perfused heart of rabbit (Hagen, 1939), isolated strips of ventricular musculature of the turtle (Wedd, 1939), and striated muscle (Cattell and Goodall, 1937).

As a summarizing statement it can be said that after therapeutic dose of cardiac glycosides the potassium content in the heart either decreases, increases, or remains the same, while after toxic dose there is a definite decrease of this electrolyte.

G. Action of Cardiac Glycosides on Adenosine Triphosphatase (ATP-ase).

If utilization of energy is impaired in cardiac failure as has been contended by Katz (1938) this may be reflected by the action of cardiac glycosides on the enzyme activity of the ATP-ase associated with actomyosin.

In extracts and homogenates of heart muscle it has been found that cardiac glycosides can cause a slight increase in the rate of dephosphorylation of ATP by calcium-activated myosin of ATP-ase (Edman, 1951). However, in cardiac muscle in the presence of ATP-ase which is not activated by calcium (Kimura and DuBois, 1947) the effect of cardiac glycosides on ATP splitting is inhibitory. This enzyme may possibly be Kielley and Meyerhof's magnesium-activated ATP-ase (1948).

CHAPTER III

PROCEDURES AND METHODS

A. The Experimental Procedure.

In our experiments we have chosen to use therapeutic and toxic doses which were similar to those used by Dearing, Essex, Herrick, and Barnes (1943). For our single therapeutic dose, 20% of the minimal lethal dose (0.575 mg./kg. body weight) was used and for our daily therapeutic dose, 12.5% of the minimal lethal dose (MLD) was administered. As a single toxic dose, 80% of the MLD was selected and as a daily toxic dose, 50% of the MLD was given.

The digitoxin was obtained from Abbott Laboratories in the form of a powder. To make the necessary solution the powder was first dissolved in alcohol and then brought to volume with water.

In a preliminary group of experiments animals were studied over a period of 7 days, one group of dogs receiving a single therapeutic dose and the other, daily therapeutic doses. The injections were given into the saphenous vein at a very slow rate. The animals were sacrificed in both groups in 2, 3, 4, and 7 days respectively after the first injection. It was decided

that 2 and 4 days should be adequate in ascertaining definitely the effect of digitoxin on the chemical constituents of the heart.

In the major group of studies two series of experimental animals were studied namely, one receiving therapeutic doses and the other toxic doses. Each series was divided into a singly and daily injected groups. In each group, animals were sacrificed after 2 and 4 days. Control animals were taken at suitable intervals.

Electrocardiograms were taken before and after the administration of digitoxin. Upon analysis, the heart rate is either increased, decreased, or remains the same, also variable T wave changes were observed. Both these observations are in accordance with the literature (Dearing et. al., 1943). Since the results were so variable in the dog, EKG recordings were not used in the evaluation of drug effectiveness.

At the appropriate time, the dogs were anesthetized with 6.5% nembutal (5 ml./kg.) intraperitoneally. The trachea was opened and artificial respiration applied. The chest was opened, the pericardium split, and the heart was excised very rapidly and dropped into a beaker filled with a dry ice-ether freezing mixture.

B. Preparation of the Heart Samples for Analysis.

After thorough freezing of the heart, samples of the

left and right ventricle were chipped off and kept in another beaker containing the freezing mixture. All samples were cleared of excessive blood, papillary muscle, and epicardium.

About 1.5 gram samples of each ventricle were weighed out for glycogen analysis. These samples were placed into a test tube containing 5 ml. of 30% potassium hydroxide (KOH). Another gram sample to be used for sodium and potassium determinations was placed into a digesting tube and stoppered for later analysis.

The sample for the analysis of phosphate fractions, weighing about 2.5 to 3.0 grams, was placed in a precooled morter and pestle containing a small amount of 5% trichloracetic acid (TCA) and was crushed thoroughly. The crushed tissue was filtered through a phosphate-free filter paper into a cold 50 ml. graduated glass-stoppered cylinder. The morter was washed several times with TCA. The filter paper was also repeatedly washed until a volume of the filtrate of about 45-50 ml. was reached.

The filtrate was mixed well by shaking, the volume recorded and an aliquot of 5 ml. was taken for lactic acid analysis, transferred to a test tube and placed in the refrigerator for later analysis. The remaining extract was then neutralized to phenolpthalein with 30% KOH, brought to a volume of 50 ml. and stored in the refrigerator.

Since the phosphate fractions are so labile, it is

important that the preparation of the sample be completed quickly, and to prevent the hydrolysis of phosphocreatine (PC), all operations were carried out in a deep freeze at 0°C.

C. Chemical Determinations.

1. Phosphate Compounds.

The following phosphate determinations were carried out: inorganic phosphate (IP), the phosphorus of phosphocreatine (PC), and the phosphorus of adenosine polyphosphate (APP). The determination of inorganic phosphate is based on the color reaction of Fiske and Subbarow (1929). The production of a stable color when molybdate is reduced in the presence of acid is the basis for this reaction.

Inorganic phosphate is precipitated as the calcium salt. To 0.2 ml. of a 10% $CaCl_2$, 1.0 ml. of the extract is added and mixed well. After standing 5 minutes at room temperature, the sample is centrifuged for 15 minutes, drained, and 0.5 ml. of 2.5% $CaCl_2$ is added. The precipitate is carefully mixed, recentrifuged and the supernatent liquid drained off. Five drops of 10% HCl dissolves the precipitate and the solution is diluted with distilled water to 3 ml.

The following color reagents are added in order: 0.4 ml. of 10 N H_2SO_4, 0.8 ml. of 2.5% ammonium molybdate, 0.4 ml. of the reducing agent. The contents are brought to a volume of 10 ml., mixed, and after the full development of the color (10

minutes), the samples are read against a blank in the spectrophotometer at a wave length of 660 millimicrons.

The phosphorus of PC is determined, using the method of Fiske and Subbarow (1929), by subtracting the value for the IP from a value obtained as follows: 1.0 ml. of the extract is brought to 3.0 ml., 0.4 ml. of 10 N H_2SO_4 and 0.6 ml. of 2.5% ammonium molybdate are added respectively. The tube is allowed to stand at room temperature for 30 minutes in order to allow for the hydrolysis of PC. Then, 0.4 ml. of the reducing agent is added, brought to volume (10 ml.) and after the color has developed, read again as before.

The method of Lohmann (1928) is applied for the determination of the APP fraction. Again, this value is obtained by difference, this time the resulting value being the difference between the 30 minute molybdate hydrolysis and the 8 minute acid hydrolysis. The latter is carried out as follows: to 1.0 ml. of the extract, 1.0 ml. of a 2 N HCl is added and placed in a boiling water bath for 8 minutes. The tubes are cooled to room temperature and the following are added: 1.0 ml. of water, the same amounts of the color reagents used previously, bringing again to a final volume of 10 ml. The solution is then read in the spectrophotometer as before.

2. Glycogen.

The determination of glycogen as glucose is based on

the method of Good et. al. (1933), and the Nelson glucose method (1944). The tube, containing the sample in 30% KOH is now placed in a water bath for a period of 2.5 hours in order to dissolve the tissue. The contents are then filtered off, using glass wool, and brought to a volume of 20 ml. Five ml. aliquots are taken to which 5 ml. of 95% alcohol is added to precipitate the glycogen. In order to have complete precipitation, the tubes are placed in a warm water bath and slowly brought to a boil. At the point of boiling, the tubes are immediately removed, cooled, centrifuged, and drained off. Two and a half hour acid hydrolysis follows to liberate glucose from glycogen.

After neutralization of the solution, 1.0 ml. aliquots are taken for the glucose determination by the Nelson procedure. To each aliquot, 1.0 ml. of a copper reagent mixture is added, boiled for exactly 20 minutes and cooled. One ml. of arsenomolybdate reagent is added, tubes are well shaken so the formed CO_2 can escape, diluted to a final volume of 25 ml. and then are read against blank in the spectrophotometer at a wave length of 520 millimicrons. It was standard procedure to check the accuracy of the analysis by simultaneously running a set of standard glucose solutions.

3. Lactic Acid.

Lactic acid is determined according to a modified method of Miller and Muntz (1938). Two tenths of a ml. of the

filtrate is placed in a clean, dry test tube to which 7 ml. of H_2SO_4 reagent is added very slowly, shaking after the addition of the first 2 ml. (The H_2SO_4 reagent is as follows: 1.0 liter of concentrated H_2SO_4 is added to 117 ml. of water. To this mixture is added 5.6 ml. of aqueous 5% $CuSO_4$). The solution is heated in a boiling water bath for 6 minutes, then cooled to below 30°C in a running water bath. Two drops of 1.5% p-hydroxy-diphenyl solution is added and then the tubes are well shaken. This reagent is used for the development of the color reaction. The tubes are placed in a water bath at 30°C for a period of 30 minutes. This incubation must be carried out in the dark. Heating in a boiling water bath follows for a period of 90 seconds. The solutions are cooled to room temperature and read in the spectrophotometer at a wave length of 565 millimicrons.

4. Electrolytes.

The tissue samples weighed out for the electrolyte analysis are digested about 2 hours in 5 ml. of concentrated HNO_3 to a very small volume (about 1 ml.).

The digest is then diluted to a volume of 25 ml. and 10 ml. of this diluted sample is used for the determination of sodium and potassium in the Perkin Elmer flame photometer. Lithium lactate is used in all samples as an internal standard (Overman and Davis, 1947).

CHAPTER IV

RESULTS

A. The Action of Digitoxin on Myocardial Phosphate Fractions.

The concentrations of the phosphate compounds of control hearts is shown in Table I. Compared to the results of Wollenberger who also examined apical tissue of the left ventricle by the same chemical methods, the concentration of IP is 0.6 mg% lower, the PC is 2.2 mg% higher, and the APP is 2.7 mg% lower. For practical purposes, it seems that there is no marked difference in the results from the two laboratories.

In Tables II and III are reported the results on the phosphate fractions of hearts analyzed 2 and 4 days after the injection of a single therapeutic dose of digitoxin, i. e. 20% of the MLD. Tables IV and V summarize the results of the phosphate fractions of hearts analyzed 2 and 4 days following the daily injection of therapeutic doses, the strength of each injection being 12% of the MLD. None of the animals studied here died before the time the hearts were to be removed for analysis.

These results are in contradiction with those of Kimura (1940), who studied daily doses of digitoxin (12 mg./kg.) on the rat for a period of three days, with the dog heart-lung

preparation of Wollenberger (1948), and with those of Weicker (0.03-0.05 mg./kg. strophanthin) on the completely isolated Langendorff cat preparation perfused with Ringer's solution (1938). All the above investigators have reported that non-toxic doses of cardiac glycosides do not produce significant changes in PC and APP fractions.

At this point, the author wishes to stress the fact that no direct comparison can be made between the results reported here and those of the various investigators mentioned above. In those investigations, experimental conditions were different and duration of experiments with the exception of Kimura's work was rather short (several hours at most). In Kimura's work the dosage is considerably higher so that perhaps species difference is the only apparent difference in these results.

Attention should be brought to animals 9, 10, and 12 (Table II, IV, and V respectively), where PC did not change. This may be accounted for by the fact that a one month old preparation of digitoxin was injected; this indicated that the digitoxin in solution loses some of its activity on standing (Sollman, 1936). All other solutions have been prepared weekly.

It is of some interest to note that the right ventricle in almost all instances shows less change in PC than the left ventricle. With regard to the difference between the two and

four day animals in both groups, the data are insufficient to detect any significant trend.

In Tables VI and VII are summarized the values of the phosphate fractions of experimental animals, to which a single toxic dose of digitoxin, 80% of MLD, has been administered. Two and four days after the injection the animals have been sacrificed. In this group 9 out of 19 dogs died within 24 hours after a single injection.

Again the PC fraction shows a marked decrease; APP and IP show an insignificant increase or no change at all. These results are in accordance with those of Chen and co-workers on the isolated rabbit heart (1947), and with those of Wollenberger (1948), who found the same effect of digitalis drugs in toxic doses in the dog heart-lung preparation as well as in the intact animals. Weicker (1935) has reported that contracture-producing doses of strophanthin cause a greater loss of APP than PC in the isolated cat heart perfused with Ringer's solution.

Results of toxic injections of digitoxin given 2 days are reported in Table VIII. IP is increased markedly, APP slightly, while PC is decreased to almost a half of the normal value. In this group of animals, 4 out of 9 animals died after the second injection.

It has been impossible to maintain dogs on daily toxic injections for 4 days (lost 12 out of 13 dogs). The mortality

being so great it was decided to discontinue this group from the experimental series. Evidently the action of the total amount of digitoxin administered is cumulative and was too toxic for the animals to survive.

In summary (see Table IX) it can be stated that in all the experimental groups PC is significantly decreased; there appears to be a tendency for IP to increase and APP does not seem to be decreased at all even by toxic doses of digitoxin.

B. The Action of Digitoxin on Myocardial Glycogen and Lactic Acid.

Control values for glycogen and lactic acid are reported in Table X. These values compare with those in the literature (Visscher and Mulder, 1930; Cruickshank and Shrivastava, 1930). It should be noted that a wide range of values is possible for glycogen content. Single therapeutic dose of digitoxin administered to dogs which are sacrificed 2 or 4 days after injection, produced no change in glycogen and slight increase in lactic acid (Table XI and XII). Therapeutic doses of digitoxin given daily for 3 and 4 days also do not alter the glycogen and lactic acid content markedly (Tables XIII and XIV). If, however, one ignores experiments 9, 10, and 12, which were injected with an old digitoxin solution, it appears that there is a tendency for glycogen to decrease and lactic acid to increase. The reported rise in lactic acid is in accordance with all the investi-

gators mentioned previously and glycogen decrease is consistent with this observation.

It was surprising to observe that in addition to the increase in lactic acid concentration also glycogen content (Table XV) rises significantly in animals 2 days after a single toxic injection of digitoxin. The animals in this group have been so sick that they were unable to eat throughout the entire experimental period. It has been reported by Cruickshank (1936), and Lackey (1947) that fasting will cause an increase in cardiac glycogen. On the other hand, the glycogen and lactic acid results from animals living 4 days after a single toxic injection show no marked change in either constituent (Table XVI). The animals in this group were apparently eating after an initial period of fasting. Animals that received repeated toxic injections for 2 days showed no change (Table XVII).

Summarizing all the results on glycogen and lactic acid (Table XVIII) it appears that digitoxin tends to cause an increase in lactic acid and a decrease in glycogen if the effects of fasting are taken into account.

C. <u>The Action of Digitoxin on Myocardial Electrolytes.</u>

Control values for sodium and potassium are found in Table XIX. These values are comparable with those found in the literature (Boyer and Poindexter, 1940; Wood and Moe, 1942).

Sodium remains more or less constant (Table XX-XXIII

inclusive) in the groups given single or daily injection of therapeutic doses. Potassium tends to rise, reaching a statistically significant increase in the right ventricle in the group of repeated injections of therapeutic doses (Table XXII). With repeated injections of digitoxin for 4 days the potassium content in the left ventricle decreases (Table XXIII), while the concentration in the right ventricle increases. We are at a loss to explain this change. Toxic doses (see below) also cause an increase in tissue potassium.

The increase in potassium content is in agreement with Hagen's report (1939), who has found that therapeutic doses of digitalis cause a uniform and significant rise in the potassium content of isolated rabbit hearts, whereas toxic doses produce a decrease in potassium. Boyer and Poindexter (1940) also have reported that a therapeutic dose of digitalis increase the potassium content of the hearts of intact cats.

Single toxic injection over 2 and 4 days produces the following changes: sodium shows a marked depletion in its content while there appears to be a tendency for potassium to increase (Table XXIV and XXV). Similar results were observed when toxic doses were given daily for 2 days (Table XXVI).

Summarizing the results of electrolytes (Table XXVII), it can be said that in all groups sodium tends to decrease while potassium seems to increase with the exception of the group in

which the animals received repeated injections of therapeutic doses of digitoxin for 4 days. There, in the left ventricle, potassium concentration decreases.

TABLE I. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF NORMAL DOGS.

DOGS NO.	WEIGHT Kg.	SEX	IP		PC		APP	
			RV	LV	RV	LV	RV	LV
1	14	M	19.4	15.4	21.2	20.6	44.0	42.8
2	14	M	14.8	16.2	19.4	12.2	29.5	22.1
3	15	M	27.2	26.4	8.6	13.6	33.9	40.4
8	20	M	37.4	34.0	14.6	10.0	32.9	37.2
13	14	M	25.5	28.2	13.0	11.1	38.9	30.9
16	13	M	29.4	38.1	13.2	11.8	36.1	25.4
19	15	M	29.3	29.1	9.0	11.7	34.2	35.0
22	13	M	39.0	32.3	13.9	12.5	34.5	28.8
25	14	M	28.2	25.9	11.1	7.7	35.1	35.8
28	16	M	30.5	24.3	11.0	11.7	34.3	36.7
33	15	M	15.6	26.0	12.0	15.5	35.2	32.1
41	15	M	26.1	27.7	13.2	11.4	32.7	34.0
45	17	F	29.5	26.0	10.9	13.6	30.4	30.8
Mean			25.8	26.2	13.2	12.5	34.9	33.3
S. E. of Mean			±1.7	±1.3	±1.0	±0.8	±1.0	±1.6

IP. . . . . Inorganic Phosphate.
PC. . . . . Phosphocreatine.
APP . . . . Adenosine Polyphosphate.
RV. . . . . Right Ventricle.
LV. . . . . Left Ventricle.
mg%. . . . Milligrams of phosphorus per 100 grams of tissue.

TABLE II. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF DOGS RECEIVING A SINGLE THERAPEUTIC DOSE. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	WEIGHT Kg.	Mg/DOSE	SEX	IP		PC		APP	
				RV	LV	RV	LV	RV	LV
4	12	1.4	F	28.8	23.2	4.8	4.6	43.7	42.3
9	18	2.1	M	30.5	34.1	10.7	8.3	39.4	40.1
14	15	1.8	M	33.9	29.4	4.1	1.6	40.3	38.7
38	11	1.3	M	26.7	25.4	4.1	3.2	36.6	32.2
39	14	1.6	M	27.8	34.1	7.5	3.8	39.1	35.0
Mean.				29.5	29.2	6.2	4.1	39.8	37.6
S. E. of Mean.				±1.2	±2.1	±1.2	±1.1	±1.1	±1.7
p Value.						<.01	<.01	.01	

TABLE III. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF DOGS RECEIVING A SINGLE THERAPEUTIC DOSE. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	WEIGHT Kg.	Mg/DOSE	SEX	IP		PC		APP	
				RV	LV	RV	LV	RV	LV
6	18	2.1	M	33.2	34.1	4.0	2.0	38.0	34.2
11	15	1.8	M	30.0	32.3	6.0	0.0	37.2	32.5
17	15	1.8	M	30.4	31.2	8.5	3.1	33.3	36.4
34	11	1.3	M	33.3	32.2	8.9	2.3	25.3	28.9
35	10	1.1	M	24.4	23.5	8.0	3.4	31.1	28.3
Mean.				30.2	30.6	7.1	2.2	32.9	32.0
S. E. of Mean.				±1.4	±1.8	±0.9	±0.3	±2.2	±1.4
p Value.						<.01	<.01		

p Value refers to the probability that the samples were drawn from the same population as the control hearts and was evaluated by Fisher's t test.

TABLE IV. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF DOGS RECEIVING DAILY THERAPEUTIC DOSES. DURATION OF EXPERIMENT, TEN DAYS.

DOG NO.	WEIGHT Kg.	Mg/DOSE	SEX	IP		PC		APP	
				RV	LV	RV	LV	RV	LV
5	14	1.0	M	36.2	39.7	5.2	2.9	44.0	42.6
10	14	1.0	F	29.7	27.5	11.8	10.7	36.5	37.4
15	14	1.0	M	37.8	25.9	0.9	2.7	36.8	30.0
36	9	0.6	M	34.8	28.3	5.6	3.9	32.7	30.5
37	12	0.9	M	37.5	39.5	7.6	5.6	32.0	30.4
Mean.				35.0	32.2	6.2	5.2	36.4	34.2
S. E. of Mean.				±1.4	±3.0	±1.7	±1.5	±0.02	±2.4
p Value.				.01	.01	<.01	.03		

TABLE V. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF DOGS RECEIVING DAILY THERAPEUTIC DOSES. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	WEIGHT Kg.	Mg/DOSE	SEX	IP		PC		APP	
				RV	LV	RV	LV	RV	LV
7	12	0.9	M	26.8	30.0	8.0	4.2	37.7	40.7
12	9	0.6	F	25.1	39.6	14.6	0.0	40.5	35.6
18	12	0.9	M	30.2	28.3	5.3	5.1	32.0	39.4
42	11	0.8	F	25.7	39.8	7.0	1.6	35.4	24.2
43	11	0.8	M	27.2	28.5	5.3	2.6	30.0	28.9
Mean.				27.0	33.2	8.0	2.7	35.1	31.1
S. E. of Mean.				±0.9	±2.6	±1.7	±0.6	±1.9	±2.9
p Value.					.02	.03	<.01		

TABLE VI. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF DOGS RECEIVING A SINGLE TOXIC DOSE. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	WEIGHT Kg.	Mg/DOSE	SEX	IP		PC		APP	
				RV	LV	RV	LV	RV	LV
20	14	6.3	M	30.2	32.6	4.8	0.0	40.5	33.4
24	16	7.5	M	28.5	32.5	4.3	2.6	38.3	37.8
26	11	4.9	M	37.4	31.7	3.3	5.5	36.2	36.4
30	11	4.9	M	39.2	29.1	4.0	3.8	39.1	37.7
40	10	4.6	M	29.3	29.2	4.0	3.7	39.3	37.8
Mean.				30.9	31.0	5.2	3.1	38.6	36.6
S. E. of Mean.				±1.6	±0.8	±0.6	±0.6	±0.7	±0.8
p Value.					.05	<.01	<.01	.05	

TABLE VII. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF DOGS RECEIVING A SINGLE TOXIC DOSE. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	WEIGHT Kg.	Mg/DOSE	SEX	IP		PC		APP	
				RV	LV	RV	LV	RV	LV
23	12	5.6	M	27.0	26.4	5.2	7.0	38.0	35.8
29	9	4.2	M	27.6	22.0	8.9	8.7	35.2	34.1
31	13	6.1	M	29.2	27.4	6.3	5.9	34.7	35.0
32	10	4.6	M	27.7	28.8	6.3	4.6	35.1	36.3
44	16	7.5	M	25.4	28.1	8.3	8.6	29.9	33.7
Mean.				27.4	26.5	7.0	6.9	34.2	34.9
S. E. of Mean.				±0.6	±1.2	±2.2	±0.8	±1.3	±0.5
p Value.						<.01	<.01		

TABLE VIII. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF DOGS RECEIVING DAILY TOXIC DOSES. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	WEIGHT Kg.	Mg/DOSE	SEX	IP		PC		APP	
				RV	LV	RV	LV	RV	LV
21	8	2.2	M	39.6	32.0	10.6	4.9	35.4	39.7
27	14	3.9	M	33.9	28.0	2.3	4.9	34.6	40.0
46	15	4.2	M	29.8	33.3	6.3	6.4	38.0	38.1
47	14	3.9	M	34.7	31.9	8.2	5.3	32.3	43.6
48	9	2.6	M	27.9	33.6	9.0	4.6	30.1	37.5
Mean.				33.2	31.8	7.6	5.2	34.0	39.8
S. E. of Mean.				±2.4	±1.1	±1.3	±0.3	±1.3	±1.1
p Value.				.05	.03	<.01	<.01		<.01

TABLE IX. SUMMARY OF THE MEANS OF PHOSPHATE CONCENTRATIONS (mg%) IN DIGITOXIN TREATED AND CONTROL ANIMALS.

	IP		PC		APP	
	RV	LV	RV	LV	RV	LV
CONTROL MEAN	28.8	26.2	13.2	12.6	34.9	33.3
SINGLE THERAPEUTIC DOSE, TWO DAYS	29.5	29.2	6.2 (<.01)	4.1 (<.01)	39.8 (.01)	37.6
SINGLE THERAPEUTIC DOSE, FOUR DAYS	30.2	30.6	7.1 (<.01)	2.2 (<.01)	32.9	32.0
DAILY THERAPEUTIC DOSES, TWO DAYS	35.0 (.01)	32.2 (.01)	6.2 (<.01)	5.2 (<.03)	36.4	34.2
DAILY THERAPEUTIC DOSES, FOUR DAYS	27.0	33.2 (.02)	8.0 (.03)	2.7 (<.01)	35.1	31.1
SINGLE TOXIC DOSE, TWO DAYS	30.9	31.0 (.05)	5.2 (<.01)	3.1 (<.01)	38.6 (.05)	36.6
SINGLE TOXIC DOSE, FOUR DAYS	27.4	26.5	7.0 (<.01)	6.9 (<.01)	34.2	34.9
DAILY TOXIC DOSES, TWO DAYS	33.2 (.05)	31.8 (.03)	7.6 (<.01)	5.2 (<.01)	34.0	39.8 (<.01)

p Value, which is indicated within the parenthesis, refers to the probability that the samples were drawn from the same population as the control hearts and was evaluated by Fisher's t test.

TABLE X. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF NORMAL DOGS.

DOG NO.	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
1	786	792	21.6	18.0
2	767	463	14.2	15.3
3	1097	867	12.5	13.8
8	894	867	14.7	19.5
13	1038	921	18.0	12.0
16	770	582	12.9	18.8
19	732	560	13.4	14.4
22	851	695	15.6	17.4
25	989	753	18.0	17.0
28	792	626	12.5	13.1
33	760	613	12.2	10.5
41	717	647	12.2	14.0
45	645	563	12.3	10.2
Mean.	837	689	14.4	14.9
S. E. of Mean.	±76	±39	±0.81	±0.84

TABLE XI. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF DOGS RECEIVING A SINGLE THERAPEUTIC DOSE. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
4	653	574	36.9	35.2
9	1017	804	12.8	17.3
14	838	790	15.0	19.5
38	860	896	14.8	21.0
39	898	752	13.8	24.8
Mean.	853	763	18.7	23.5
S. E. of Mean. . .	±59	±38	±4.5	±3.1
p Value.				<.01

TABLE XII. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF DOGS RECEIVING A SINGLE THERAPEUTIC DOSE. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
6	371	377	14.3	13.5
11	697	654	14.6	34.3
17	846	779	17.3	24.0
34	972	721	18.7	14.4
35	880	635	15.5	44.5
Mean.	753	633	16.8	26.2
S. E. of Mean. . .	±105	±69	±2.7	±5.9
p Value.				.01

TABLE XIII. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF DOGS RECEIVING DAILY THERAPEUTIC DOSES. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
5	561	454	17.6	32.0
10	1220	987	21.5	10.5
15	779	507	21.6	24.0
36	652	506	10.5	10.8
37	718	517	18.8	20.5
Mean.	764	594	18.0	17.5
S. E. of Mean. . .	±33	±98	±2.0	±4.1
p Value.				

TABLE XIV. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF DOGS RECEIVING DAILY THERAPEUTIC DOSES. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
7	510	372	15.2	21.9
12	1015	975	6.8	12.9
18	785	844	17.1	18.5
42	825	750	14.5	27.7
43	650	579	10.3	13.6
Mean.	757	704	12.7	18.9
S. E. of Mean. . .	±64	±104	±1.8	±2.7
p Value				

TABLE XV. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF DOGS RECEIVING A SINGLE TOXIC DOSE. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
20	942	862	14.6	28.3
24	903	963	14.4	17.4
26	1091	1143	17.6	21.5
30	1061	1064	18.5	18.9
40	989	946	18.0	17.4
Mean.	997	994	16.6	20.7
S. E. of Mean. . .	±36	±30	±0.87	±2.0
p Value.		<.01		.01

TABLE XVI. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF DOGS RECEIVING A SINGLE TOXIC DOSE. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
23	736	575	12.7	13.1
29	680	439	12.6	9.0
31	644	501	13.4	11.5
32	628	613	12.6	13.1
44	654	592	11.7	11.1
Mean.	678	544	12.4	11.5
S. E. of Mean. . .	±29	±32	±0.3	±0.7
p Value.		.05		.05

TABLE XVII. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF DOGS RECEIVING DAILY TOXIC DOSES. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
21	956	925	18.7	22.1
27	1043	904	21.6	14.2
46	896	683	15.6	13.9
47	957	697	15.4	18.5
48	571	521	12.7	15.5
Mean.	884	746	16.4	16.8
S. E. of Mean. . .	±76	±75	±1.4	±1.5
p Value.				

TABLE XVIII. SUMMARY OF THE MEANS OF GLYCOGEN AND LACTIC ACID CONCENTRATIONS (mg%) IN DIGITOXIN TREATED AND CONTROL ANIMALS.

	GLYCOGEN		LACTIC ACID	
	RV	LV	RV	LV
CONTROL MEAN	837	689	14.4	14.9
SINGLE THERAPEUTIC DOSE, TWO DAYS	853	765	16.7	23.5 (<.01)
SINGLE THERAPEUTIC DOSE, FOUR DAYS	755	633	16.5	26.2 (.01)
DAILY THERAPEUTIC DOSE, TWO DAYS	784	594	18.0	17.5
DAILY THERAPEUTIC DOSE, FOUR DAYS	757	704	12.7	18.9
SINGLE TOXIC DOSE, TWO DAYS	997	994 (<.01)	16.6	20.7 (.01)
SINGLE TOXIC DOSE, FOUR DAYS	672	544 (.05)	13.4	11.5 (.05)
DAILY TOXIC DOSE, TWO DAYS	884	746	16.4	16.8

p Value, which is indicated within the parenthesis, refers to the probability that the samples were drawn from the same population as the control hearts and was evaluated by Fisher's t test.

TABLE XIX. CONCENTRATIONS (meq./Kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF NORMAL DOGS.

DOG NO.	SODIUM		POTASSIUM	
	RV	LV	RV	LV
1	39.7	38.1	64.4	63.7
2	37.0	37.7	70.1	69.3
3	44.0	36.3	62.1	65.3
8	50.0	44.2	76.3	74.2
13	49.3	34.8	69.2	53.0
16	33.4	36.3	54.3	69.2
19	46.2	30.2	77.7	59.4
22	38.8	42.1	55.0	66.0
25	47.8	30.0	71.5	50.0
28	45.5	37.4	78.8	58.5
33	46.8	53.0	71.8	76.3
41	51.5	55.4	63.2	58.1
45	45.7	38.6	76.7	69.5
Mean.	44.3	40.3	68.5	64.0
S. E. of Mean. . .	±1.5	±2.1	±2.2	±1.8

TABLE XX. CONCENTRATIONS (meq./Kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF DOGS RECEIVING A SINGLE THERAPEUTIC DOSE. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	SODIUM		POTASSIUM	
	RV	LV	RV	LV
4	38.8	40.6	79.5	85.5
9	45.2	35.0	83.6	75.7
14	23.9	37.5	47.9	52.8
38	50.1	51.1	73.8	75.0
39	48.6	37.6	73.9	75.5
Mean.	41.3	40.4	71.7	72.9
S. E. of Mean. . . .	±2.7	±1.1	±1.9	±5.3
p Value.				

TABLE XXI. CONCENTRATIONS (meq./Kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF DOGS RECEIVING A SINGLE THERAPEUTIC DOSE. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	SODIUM		POTASSIUM	
	RV	LV	RV	LV
6	35.0	33.4	57.5	68.5
11	53.5	54.2	69.8	83.8
17	39.2	14.9	75.0	40.0
34	32.2	47.5	71.7	85.8
35	49.0	49.3	80.0	77.5
Mean.	41.8	39.8	70.8	71.1
S. E. of Mean. . . .	±4.1	±7.0	±3.7	±6.3
p Value.				

TABLE XXII. CONCENTRATIONS (meq./Kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF DOGS RECEIVING DAILY THERAPEUTIC DOSES. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	SODIUM		POTASSIUM	
	RV	LV	RV	LV
5	39.9	36.2	93.5	88.0
10	46.4	22.5	76.4	40.5
15	35.0	31.7	78.2	59.9
36	47.1	50.5	78.0	78.0
37	47.5	53.7	81.4	84.1
Mean.	43.4	38.9	81.5	70.1
S. E. of Mean. . . .	±2.4	±5.8	±3.1	±8.8
p Value.			.01	

TABLE XXIII. CONCENTRATIONS (meq./Kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF DOGS RECEIVING DAILY THERAPEUTIC DOSES. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	SODIUM		POTASSIUM	
	RV	LV	RV	LV
7	52.5	34.6	90.5	51.5
12	56.0	36.0	74.5	48.7
18	52.5	31.3	73.1	56.4
42	28.8	26.5	81.1	55.0
43	51.3	40.9	85.0	54.5
Mean.	48.2	33.8	80.8	53.2
S. E. of Mean. . . .	±4.8	±2.4	±5.9	±1.3
p Value.			.05	.05

TABLE XXIV. CONCENTRATIONS (meq./Kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF DOGS RECEIVING A SINGLE TOXIC DOSE. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	SODIUM		POTASSIUM	
	RV	LV	RV	LV
20	30.6	41.5	56.9	78.7
24	26.0	26.9	70.0	65.0
26	14.2	24.7	68.9	69.3
30	32.0	31.5	72.5	80.0
40	34.0	52.6	71.5	76.0
Mean.	27.3	31.4	68.4	73.8
S. E. of Mean.	±3.5	±3.9	±2.4	±2.8
p Value.	.01	.05		.05

TABLE XXV. CONCENTRATIONS (meq./Kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF DOGS RECEIVING A SINGLE TOXIC DOSE. DURATION OF EXPERIMENT, FOUR DAYS.

DOG NO.	SODIUM		POTASSIUM	
	RV	LV	RV	LV
23	42.2	40.0	75.0	84.1
29	42.7	35.8	83.0	46.7
31	41.8	40.6	83.6	75.2
32	46.6	43.5	82.5	75.0
44	66.0	61.9	70.0	82.4
Mean.	47.8	44.3	78.2	72.7
S. E. of Mean. . . .	±4.7	±4.4	±2.7	±6.7
p Value.			.05	.05

TABLE XXVI. CONCENTRATIONS (meq./Kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF DOGS RECEIVING DAILY TOXIC DOSES. DURATION OF EXPERIMENT, TWO DAYS.

DOG NO.	SODIUM		POTASSIUM	
	RV	LV	RV	LV
21	39.7	23.4	81.2	44.6
27	23.7	22.8	35.4	40.3
46	35.4	37.0	87.5	84.0
47	34.7	26.4	92.8	83.5
48	34.6	36.0	74.0	84.0
Mean.	33.6	29.1	73.7	67.3
S. E. of Mean.	±2.6	±3.0	±10.5	±10.0
p Value.	<.01	<.01		

TABLE XXVII. SUMMARY OF THE MEANS OF SODIUM AND POTASSIUM CONCENTRATIONS (meq./Kg.) IN DIGITOXIN TREATED AND CONTROL ANIMALS.

	SODIUM		POTASSIUM	
	RV	LV	RV	LV
CONTROL MEAN	44.3	40.3	66.5	64.0
SINGLE THERAPEUTIC DOSE, TWO DAYS	41.3	40.4	71.7	72.9
SINGLE THERAPEUTIC DOSE, FOUR DAYS	41.8	39.8	70.8	71.1
DAILY THERAPEUTIC DOSE, TWO DAYS	43.4	38.9	81.5 (.01)	70.1
DAILY THERAPEUTIC DOSE, FOUR DAYS	46.2	33.8	80.8 (.05)	53.2 (.05)
SINGLE TOXIC DOSE, TWO DAYS	27.3 (.01)	31.4 (.05)	66.4	73.8 (.05)
SINGLE TOXIC DOSE, FOUR DAYS	47.8	44.3	78.2 (.05)	72.7 (.05)
DAILY TOXIC DOSE, TWO DAYS	33.6 (<.01)	29.1 (<.01)	73.7	67.3

p Value, which is indicated within the parenthesis, refers to the probability that the samples were drawn from the same population as the control hearts and was evaluated by Fisher's t test.

CHAPTER V

DISCUSSION

A. Effectiveness of Administered Digitoxin.

Regardless of the thoroughness with which an experiment is conducted, there may be some question as to whether the desired experimental influence has been effected. For example, even though an administered drug may be procured from a reliable firm, the investigator may inadvertently inactivate the drug prior to use. We have, in fact, observed these results with a month old solution of digitoxin in a few experiments.

We feel reasonably certain that effective concentrations of digitoxin were used in this study. This is supported by our care in handling the drug prior to administration, the measured changes in metabolism, and the high mortality when toxic levels of the drug were employed.

B. The Action of Digitoxin on Energy-Rich Phosphate Metabolism.

In order to elucidate the mechanism of action of cardiac glycosides, it is important to determine the change in energy metabolism that these drugs may cause in the living organism. One approach to this problem is to analyze the content of certain energy-rich phosphate compounds, adenosine polyphosphate

(APP) and phosphocreatine (PC). (Since the analytical method employed in this study recovers the labile P of both adenosine triphosphate and diphosphate, the term polyphosphate is used to refer to both of these compounds.) These compounds are of primary importance in metabolism since (1) the sum total of the reactions which degrade the foodstuffs are directed at the synthesis of these compounds and (2) these compounds are directly used as the form of chemical energy which is usable in a number of physiological processes, such as muscular contraction.

The major finding in the investigations reported in this thesis is that cardiac glycosides decrease the content of PC at doses short of toxicity. Associated with this observation is the increase in IP in many instances and the lack of change in APP, except at toxic doses.

The decrease in the level of energy-rich phosphate may be due to a number of alternative possibilities. Digitoxin may act by simply increasing the rate of utilization while the rate of synthesis remains relatively unchanged. A second possibility is that the rate of synthesis may be inhibited while the rate of utilization remains relatively unchanged. Although it is not impossible for both rates to be increased or decreased and for one rate to be changed to a different degree than the other, these alternatives will not be used unless simpler explo-

nations do not suffice.

Despite the uncertainty of the situation, certain reasonable assumptions may be invoked to choose the most likely explanation. Decrease in the rate of synthesis is unlikely in view of the fact that the cardiac glycosides increase oxygen consumption and glucose and lactate utilization (Peters and Visscher, 1936; Gremel, 1940; Mel'nikova, 1942). However, the rate of synthesis of energy-rich phosphate may be depressed even in the face of an increased oxygen consumption if the coupling of oxidation and phosphorylation is impaired. High concentrations of cardiac glycoside do not uncouple oxidative phosphorylation according to Hermann (1950), and Wollenberger (1951). If these results are correct, synthesis seems to be unimpaired by cardiac glycosides and, in fact, may be increased in its presence.

On the other hand, the utilization of energy-rich phosphates may be increased by cardiac glycosides. The evidence in support of this is concerned with studies on the enzyme preparations of heart muscle. Cardiac glycosides have been shown to produce a slight increase in the rate of dephosphorylation of ATP by calcium-activated myosin-ATPase (Edman, 1951). It has thus been suggested by Hegglin (1951) that this may be the manner in which the failing heart is benefited by the cardiac glycosides.

Other studies employing the soluble magnesium-activated, calcium-inhibited ATPase (Kielley and Meyerhof, 1948) or a phosphatase which is not activated by calcium (Kimura and DuBois, 1947) indicate the action of the cardiac glycosides on ATP dephosphorylation is inhibitory (Kimura and DuBois, 1947). These studies need not be contradictory. The calcium-activated ATPase may be associated with myosin and the contraction process. The calcium-inhibited ATPase may be related with intracellular dephosphorylations without which the metabolic machinery cannot proceed constantly in the relatively resting state. That is, without the constant breakdown and resynthesis of ATP, the absence of inorganic phosphate may produce a Harden-Young effect which could inhibit metabolism (Meyerhof, 1949). The action of digitalis on both of these forms of ATPase could have important adaptive significance. It may also mean that in cardiac failure the action of calcium-inhibited ATPase may be greater than normal.

For these reasons, then, the action of digitoxin in these experiments may be considered to be on increasing the rate of utilization of energy-rich phosphates. These conclusions are similar to those of Wollenberger (1951) who, however, did not observe any change in energy-rich phosphate levels with non-toxic doses of digoxin or ouabain. His interpretations were based on changes which were observed in hearts given toxic amounts of the

drug. With toxic concentrations, the interpretation of the results is difficult since the change in energy-rich phosphate may be a result of the fibrillation and other toxic changes, rather than of the administered drug, per se. The changes observed in the hearts given toxic amounts of the drug in Wollenberger's studies were similar to the hearts in our investigation given non-toxic as well as toxic amounts of the drug.

It is of interest to determine the probable reasons for the negative results obtained by Wollenberger. The major difference in the experiments from the two laboratories was in the time of exposure to the drug. Although it is not explicitly stated, it may be assumed from the dosage given and the rate of administration of the drug that in most of his experiments the hearts were not exposed to the cardiac glycoside for longer than one hour. In our experiments, animals were exposed to various levels of the drug for two and four days. It is quite likely that the change induced in phosphate metabolism in one hour was not sufficiently great to change the concentration of these compounds in significant amounts except when toxic doses were employed. The method of tissue removal and the part of the heart which was removed for analysis is not primarily involved since the concentrations of the normal hearts agree satisfactorily in Wollenberger's and our series. It is believed also that digoxin and ouabain that Wollenberger used and digitoxin used in this

study are not sufficiently different to account for the difference in the results.

C. The Action of Digitoxin on Glycogen and Lactic Acid.

Glycogen content tends to decrease and lactic acid concentration tends to increase following the addition of digitoxin. Our work does not appear to clear the air convincingly with regard to the equivocal results obtained thus far on these chemical compounds, since we have an insufficient number of studies to draw upon.

D. The Action of Digitoxin on Electrolytes.

With the prolonged administration of digitoxin or with toxic doses, sodium tends to decrease and potassium ion tends to increase in the heart tissue. The potassium results are confirmatory of the work of Boyer and Poindexter (1940) in intact cats, and of the work in Langendorff preparation of the rabbit heart. Our results here are not so pronounced as to strongly indicate the change in potassium which might be expected. It was somewhat surprising to note that tissue sodium decreases, in a few cases. It may be possible that digitoxin increases the utilization of ATP for the increased transport of sodium has been shown to be an active process (Conway, 1947).

E. Miscellaneous Comments.

The difference between single and daily injections of digitoxin was not sufficiently great to warrant any far reaching

conclusions. Similarly, the injection of therapeutic and toxic amounts of drug was not studied extensively enough to allow definite statements to be made. In general, the toxic doses gave more marked changes.

It is interesting to observe that the changes caused by therapeutic and toxic concentrations are in the same direction. This not necessarily is expected since toxic concentrations of any agent may cause a general picture of toxicity. However, since it is observed, it strongly suggests that the change effected in therapeutic amounts may be damaging if larger concentration of drug is given.

It is important to emphasize that our studies have not elucidated the site of action of the cardiac drugs. Since Wollenberger observed ventricular fibrillation and tachycardia before energy-rich phosphate supply is completely depleted, it may be possible that some factor which enhances the utilization of energy-rich phosphate may be entering the heart cell in greater amounts under the influence of digitoxin.

The right ventricle seemed to be relatively resistant to change in energy-rich phosphate when compared to the left ventricle. Perhaps, this is related to the relatively greater capacity of the left ventricle to utilize energy-rich phosphate compounds.

CHAPTER VI

SUMMARY

1. Single and daily intravenous injection of therapeutic and toxic doses of digitoxin were administered to adult dogs. Two and four days after the first injection, the animals were sacrificed.

2. Analysis of the frozen tissue for inorganic and energy-rich phosphates, glycogen, lactic acid, and electrolytes were carried out.

3. In all experiments, phosphocreatine content was significantly decreased.

4. With therapeutic doses, no change in APP fraction was observed and only a slight increase of IP was seen in those animals receiving daily doses of digitoxin.

5. When a single injection of toxic strength was administered, the APP fraction and IP remained normal. In the repeated injections, there was a tendency of both of the above fractions to increase.

6. Glycogen concentration tended to decrease and lactic acid significantly increased in certain experiments following the administration of digitoxin.

7. Potassium was significantly decreased after four days with daily therapeutic doses of digitoxin.

8. With toxic dose, significant diminution of sodium was observed in certain instances whereas potassium rises markedly in certain experiments.

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APPROVAL SHEET

The thesis submitted by John Rebar Jr. has been read and approved by three members of the Department of Physiology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

January 9, 1954
Date

Akiri Omachi
Signature of Advisor (FK)